SCHEME FOR AUTOSOMAL DOMINANT AND X-LINKED MENDELIAN DISEASES

CLASS	AMBRY CLASSIFICATION	CATEGORY	CRITERIA	EXCEPTIONS (NEW BASELINE CLASS)
5	Pathogenic	A 1 Needed	• Confirmed *de novo* alteration in the setting of a new disease (appropriate phenotype) in the family	• Confirmed *de novo* alteration in a novel gene with possible disease implications (4) • Likely *de novo* alteration (i.e. paternity not confirmed) with known disease association (4) • Confirmed *de novo* alteration in the setting of a discordant phenotype (3)
			• Alterations resulting in premature truncation (e.g.reading frame shift, nonsense)	• Truncation in close proximity to 3' terminus (3/4 gene specific) • LOF has not been established as mechanism of pathogenicity (e.g. MYH7) (3)
			• Other ACMG-defined mutation (i.e. initiation codon or gross deletion)	• In-frame gross deletion of a single exon not in a known protein functional domain (4) • Initiation codon that is not well conserved (4)
			• Strong segregation with disease (LOD >3 = >10 meioses)	
			• Functionally-validated splicing mutation	• In-frame skipping a single exon not in a known protein functional domain (4)
		B 4 Needed	• Significant disease association in appropriately sized case-control study(ies)	
			• Detected in individual satisfying established diagnostic critera for classic disease without a clear mutation	
			• Last nucleotide of exon	• When poorly conserved or *in silico* doesn't predict significant effect
			• Good segregation with disease (LOD 1.5-3 = 5-9 meioses)	
			• Deficient protein function in appropriate functional assay(s)	
			• Well-characterized mutation at same position	• Different disease causing mechanism, i.e. if other mutation affects splicing, and this particular variant is predicted to affect protein, but not slicing or nonsense vs. missense • When well characterized mutation is a proline
			• Other strong data supporting pathogenic classification	
4	Likely Pathogenic	1 Needed	• Alterations at the canonical donor/acceptor sites (+/- 1, 2) without other strong (B-level) evidence supporting pathogenicity	
		C 4 Needed	• Rarity in general population databases (dbSNP, ESP, 1000 Genomes, ExAC)	• Dependent on disease penetrance and inheritance pattern.
			• *in silico* models in agreement (deleterious) and/or completely conserved position in appropriate species	• *in silico* splicing predictions not used as independent line of evidence for last nucleotide of exon.
			• Moderate segregation with disease (at least 3 informative meioses) for rare diseases.	
			• Other data supporting pathogenic classification	
		3 of B		
		2 of B and at least 1 of C		
		1 of B and at least 3 of C		
3	VUS	Insufficient or Conflicting Evidence		
		Gross Duplications without Strong Evidence for Pathogenic or Benign		
2	Likely Benign	D 1 Needed	• Intact protein function observed in appropriate functional assay(s)	
			• Intronic alteration with no splicing impact by RT-PCR analysis or other splicing assay	
			• Other strong data supporting benign classification	
		E 2 Needed	• Co-occurence with mutation in same gene (phase unknown)	• Genes without a defined, severe biallelic phenotype (3)
			• Co-occurence with a mutation in another gene that clearly explains a pro-band's phenotype	
			• Subpopulation frequency in support of benign classification	
			• *in silico* models in agreement (benign)	
			• Does not segregate with disease in family study (genes with incomplete penetrance)	
			• No disease association in small case-control study	
			• Other data supporting benign classification	
1	Benign	F 1 Needed	• General population or subpopulation frequency is too high to be a pathogenic mutation based on disease/syndrome prevalence and penetrance	
			• Does not segregate with disease in family study (genes with complete penetrance)	
			• Internal frequency is too high to be a pathogenic mutation based on disease/ syndrome prevalence and penetrance	
			• Seen *in trans* with a mutation or in homozygous state in individual without severe disease for that gene	• Genes without a defined, severe biallelic phenotype (3)
			• No disease association in appropriately sized case-control study(ies)	
		1 of D and at least 2 of E		
		2 or more of D		
		>3 of E w/o conflicting data		
		>4 of E w/conflicting data		

The variant classification scheme is not intended for the interpretation of alterations considered epigenetic factors including genetic modifiers, multifactorial disease, or low-risk disease association alleles and may be limited in the interpretation of alterations confounded by incomplete penetrance, variable expressivity, phenocopies, triallelic or oligogenic inheritance, or skewed X-inactivation.